Supplementary Notes

One possibility is that the differences in spine turnover in previous studies are perhaps due to the differences in the subpopulation of spines visualized through either the open or the thinned-skull windows. Previous studies have compared the same cortical area first visualized through a thinned-skull, then through an open-skull window\textsuperscript{1-3}. It was found that under the same imaging setting, images of dendritic protrusions within the first 100 µm from the pial surface were highly comparable between thinned-skull (~20 µm in thickness) and open-skull windows\textsuperscript{1-3}. The same spines and filopodia can be seen through either “open-skull” or “thinned-skull” (~20 µm in thickness) windows\textsuperscript{1-3}. To further test whether or not the differences in spine turnover rates is due to differences in the subpopulation of spines detected through “open-skull” or “thinned-skull” windows, we performed experiments in which we first imaged spines under a thinned-skull window and then performed open-skull surgery immediately afterwards. Two days later, we imaged the same dendrites again under the open-skull window. We found that vast majority of spines (92.2%, 330 spines, 2 animals) first imaged through the thinned-skull window were visible under the open-skull window 2 days after surgery. Furthermore, 4.4% of new spines were formed during this period. These findings are consistent with previous results that the same population of dendritic protrusions are detected through thinned- and open-skull windows\textsuperscript{1-3} and also suggest that substantial alterations in spine dynamics under the open-skull window do not happen immediately after surgery but occurs progressively over a period of weeks.

It has been suggested that open-skull windows did not induce spine elimination and formation because spine turnover was observed more than 3 months after implantation of the imaging window\textsuperscript{4}. However, there is no quantitative data to support this argument. Thus, it is unclear from this previous study\textsuperscript{4} whether there is a time-dependent change in spine dynamics after open-skull surgery and whether the vast majority of spines are actually stable 2–3 months later. Another argument for the suitability of using open-skull windows is that
spine density in adult mice remained constant among different imaging sessions. Unfortunately, no experimental data was ever provided on spine dynamics and density in adult mice between the first day of surgery and the day of initial imaging (7–14 days later).

In previous open-skull studies, average spine density varied from ~0.24 spines/µm² to ~0.4 spines/µm² in adult mouse barrel cortex. Spine turnover also varied greatly among different cells in different animals under open-skull windows. Our findings revealed a time-dependent change in spine dynamics after open-skull surgery: a substantial loss of spines within 1–2 weeks and high spine turnover for at least 4 more weeks (Figure 1 and Supplementary Figure 1-2).

Together, these results raise the possibility that the variations of spine density and dynamics in previous studies could be due to varied effects of open-skull surgery on different cells at different ages.

A recent study has suggested that spine turnover is comparable between open-skull and thinned-skull imaging methods. The study, however, almost exclusively reported results on spine dynamics using thinned-skull imaging technique and no detailed information was given on the surgical procedure for open-skull and the duration between surgery and imaging. Nevertheless, the results obtained with thinned-skull imaging in this study are largely comparable with ours.

References